LOCALIZATION OF GLUCAGONOMAS BY PANCREATIC VEIN CATHETERIZATION AND GLUCAGON ASSAY.

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Summary

In two patients with the glucagonoma syndrome, percutaneous transhepatic portal vein puncture and pancreatic vein catheterization were performed simultaneously with inferior vena cava and abdominal aorta catheterizations. Blood samples obtained from the cava, aorta and portal branches were assayed for glucagon. In both cases, clear cut arterio-venous glucagon differences allowed preoperative localization of the tumors. A comparison with other localization techniques such as scintiscan, ultrasound, arteriography and pancreatic phlebography showed the present method to be superior. Postoperatively the same investigations were performed revealing arterial and venous glucagon levels comparable to the levels measured in a reference group of three patients without glucagonomas. The tripple catheterization technique is advocated for all patients with clinical and laboratory findings that suggest endocrine pancreatic tumors.

A characteristic syndrome associated with glucagon secreting tumors of the pancreas was first recognized in 1974 (15). Until now only a few glucagonomas have been reported in the literature. The tumors have often been diagnosed late when they have become quite large or have already metastasized (10, 14, 15, 16, 21, 24). Despite slow growth, the prognosis is poor if total resection is impossible (15, 19). Recognition of the glucagonoma syndrome and radioimmunoassay-technique for glucagon facilitate early diagnosis. Until now localization of endocrine tumors has been done by a variety of methods, such as palpation, scintiscan, ultrasound, angiography, laparoscopy or exploratory laparotomy. Since the method currently considered best for preoperative localization of endocrine tumors - arteriography - has an accuracy of 40-60% (3, 22) there is a need for more accurate diagnostic procedures. We have earlier reported on the localization of endocrine tumors by pancreatic vein catheterization (4) with blood sampling for hormone assay (7, 8, 9).

The purpose of the present study was:

1. To examine preoperatively if a pathologically raised, localized, pancreatic, arterio-venous glucagon difference could be found in patients with the glucagonoma syndrome.
2. To examine peroperatively if the arteriovenous glucagon difference combined with the knowledge of the pancreatic vein anatomy could be used for localization of the tumors.
3. To compare results obtained by conventional localization procedures - palpation, scintiscan, ultrasound, arteriography, phlebography and peroperative findings with those from the aorta, cava and pancreatic vein catheterizations.
4. To examine if the method could be used to check the results of operation.

Material

Two males, 62 and 69 years old, were investigated after eight hours of fasting. The investigations were performed two months before and two months after pancreatic resection for glucagonomas. The patients will be referred to as patient no. 1 and patient no. 2, respectively. Two females, 31 and 38 years old, and one male, 59 years old, with normal peripheral blood glucagon concentrations and without known tumors were catheterized (in order to exclude abdominal malignancy) after eight hours of fasting and used as a reference group.

Methods

Using local anesthesia, percutaneous transhepatic portal vein catheterization was performed (4) and at the same time inferior vena cava and

Table I. Pre- and peroperative findings in two patients with glucagonomas.

	Patient no 1	Patient nr 2
	Preoperative findings	
Palpation	Normal	Normal
Ultrasound	Normal	Normal
Scintiscan	Normal	Reduced isotop uptake in the head of the pancreas.
Selective arteriography (coeliac artery)	A tumor 4 cm in diameter in the tail of the pancreas.	Tumor in the head and the tail of the pancreas.
Portal flebography	Wide veins draining the tail of the pancreas. Contrastdefects (about 1 cm in diameter) in the dorso-lateral segment of the right liver-lobe. Metastases?	Normal
Tripple catheterization with blood sampling for hormone-assay	Glucagon hypersecretion from the tail of the pancreas (fig. 3 a). No hypersecretion from the liver.	Glucagon hypersecretion from the ventral part of the head of the pancreas (fig. 4 a). No hypersecretion from the liver.
	Peroperative findings	
Inspection	Tumor in the tail No liver metast.	Tumor in the caudal part of the head. No liver metast.
Palpation	Tumor in the tail No liver metast.	Tumor in the caudal part of the head. No liver metast.
Fine needle biopsy	Endocrine tumor in the tail. No liver metast.	Tumor in the caudal part of the head.
Histology and immunhistochemistry	Glucagonoma (some insulin-immunoreactive cells)	Glucagonoma (a few VIP-inmunoreactive cells).

abdominal aorta catheterizations were performed through the femoral vein and artery (7, 8). Blood samples were withdrawn from the abdominal aorta and from different branches of the cava including different hepatic vein branches. Subsequently one catheter was placed in the coeliac artery and one in the right hepatic vein. Thus, blood samples from these two vessels were obtained simultaneously with blood samples from different pancreatic veins obtained through a portal vein catheter. The blood samples were collected in ice-chilled tubes containing Heparin (14,3 USP Na-heparin/ml blood) and Trasylol® (500 KIU/ml blood), centrifuged at 4°C for ten minutes and stored at -20°C until radio-immunoassayed for insulin and glucagon (26, 6). The antiserum used for the glucagon assay (4317), has less than 0,5% cross reaction with highly purified GLI I (large molecular weight gut glucagon produced by F. Sundby, NOVO Research Institute, Copenhagen). There was no cross reaction with peak II GLI. All samples from the same patient were assayed in triplicates in the same run. The intraassay coefficient of variation was 6.7% at 180 pg/ml. Tissue glucagon was measured according to the method earlier described (12).

Immuncytochemistry: Tissue material was quenched to the temperature of liquid nitrogen in a propane-propylene mixture and freeze-dried. The specimens were then vapour-fixed with either diethylpyrocarbonate (DEPC) (18) or formaldehyde (1) and embedded in paraffin in vacuo. Deparaffinized sections were subjected to an indirect immunhistochemical method for the demonstration of glucagon, gastrin, insulin or pancreatic polypeptide (HPP) immunoreactivity as described in detail elsewhere (10, 11, 13). Controls were those recommended by Sternberger (23) and included the inactivation of the antisera by adding in excess their corresponding peptidic antigens. Examination of the immunfluorescence preparations was carried out in a Zeiss Standard fluorescence microscope equipped with an epilumination system and utilized a Xenon (XBO 75) lamp as light source. Filter setting were selected to give peak excitation at 490 nm.

Statistical methods used: Student's t-test for paired data and analysis of variance performed according to the F-test.

Results

Preoperative investigation revealed weight loss, glucose intolerance, necrolytic migratory erythema earlier described in detail (19) and high circulating glucagon levels in both patients. Findings obtained with palpation, ultrasound, scintiscan, arteriography, portal and pancreatic phlebography are presented in table I, fig. 1 a, b, c.

Glucagon values in the aorta, vena cava and main portal vein in patient no. 1: Glucagon levels in blood obtained from the coeliac artery, hepatic vein and the main portal vein are shown in fig. 2 a, b. The

highest values were seen in the portal venous blood and the lowest in the aorta. However, a much higher variation ($p < 0.001$) was observed preoperatively (fig. 2 a), which necessitated a logarithmic transformation of the glucagon levels (fig. 2 b), in order to perform valid tests of significance. The analyses of variance based on log. glucagon concentrations showed the differences between vascular areas to be significant ($p < 0.01$) but even between sampling times significant differences ($p < 0.01$) were observed pointing to real differences in glucagon secretion between sampling times. Nevertheless the decrease in glucagon levels from the pre- to the postoperative state in all three vascular areas was highly significant ($p < 0.001$).

Glucagon values in the aorta, vena cava and main portal vein in patient no. 2: In patient no. 2 the number of portal samples was to small to be used for analyses. However, levels measured in the hepatic vein and aorta showed preoperatively highly significant differences between vascular areas ($p < 0.001$) but no difference between sampling times. Postoperatively glucagon concentrations in the two vascular areas were significantly different ($p < 0.01$); the same was seen between sampling times. A highly significant glucagon reduction was seen in both vascular areas pre- versus postoperatively ($p < 0.001$).

Glucagon values in the pancreatic veins: Glucagon concentrations in the pancreatic veins in the reference group ranged from 56 to 2 400 pg/ml (n = 15, range 2 344, $\bar{m}$ = 620, median 490 pg/ml). The highest pancreatic vein glucagon level in the reference group, 2 400 pg/ml, was chosen as the highest normal value. Corresponding values for insulin concentrations ranged 4 - 200 microunits/ml (range = 196, $\bar{m}$ = 48.6, median = 13 microunits/ml). The highest pancreatic vein insulin concentration in the reference group 200 microunits/ml was chosen as the highest normal value.

Pancreatic venous glucagon concentrations indicated a tumor situated in the tail of the pancreas in patient no. 1 (fig. 1 a, 3 a, sample no. 9) and a tumor situated in the ventral portion of the pancreatic head in patient no. 2 (fig. 1 b, c, fig. 4 a, sample 16, 17) with partial drainage through anastomoses to the v. PSPD (fig. 1 b, c, fig. 4 a, sample 8, 9). In neither patient did glucagon concentrations in the different hepatic veins differ - fact excluding the presence of hepatic metastases. Postoperatively pancreatic vein glucagon concentrations in patient no. 1 ranged from 156 to 570 pg/ml (fig. 3 b) and in patient no. 2 from 116 to 1380 pg/ml (fig. 4 b).

All samples were assayed for insulin. The venous concentration of insulin was 330 microunits/ml in veins from the pancreatic body (fig. 4 a) in patient no. 2. Otherwise the pancreatic vein concentrations were within the range of the reference group. Peripheral insulin concentrations decreased during the preoperative investigation in patient no. 2 from 10 to 5 microunits/ml. Slightly fluctuating but normal insulin concentrations were seen during the other investigations. P-glucose

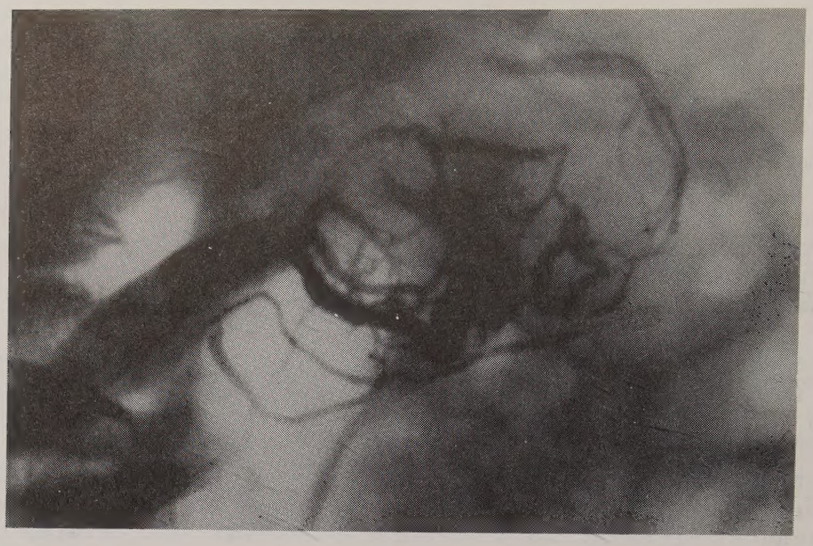

Fig. 1 a. Pancreatic phlebography of the vein draining the tumor in the tail of the pancreas (patient no. 1).

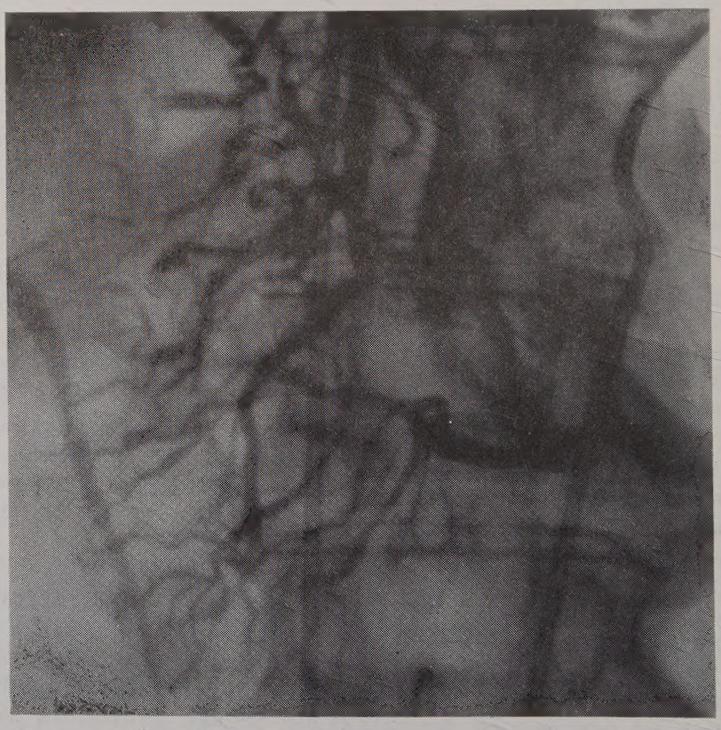

Fig. 1 b. Pancreatic phlebography of the v. PSPD with anastomoses to the tumor-draining v. ASPD (patient no. 2).

Fig. 1 c. Pancreatic phlebography of the tumor-draining v. ASPD with anastomoses to v. PSPD (patient no. 2).

from samples withdrawn during the investigations from the coeliac artery showed values slightly fluctuating but within normal range except for patient no. 2, whose glucose values increased during the investigation from 6.4 mmol/l to 8.0 mmol/l.

At surgery the venous anatomy, as revealed by the preoperative pancreatic catheterization and phlebography, could easily be confirmed. In patient no. 1 a 30 x 40 x 50 mm large, encapsulated homogenous tumor was visualized and palpated in the tail of the pancreas. Fine needle aspiration biopsy yielded cells of endocrine appearance. A distal hemipancreatectomy was performed. Other abdominal organs (including liver and lymphnodes) appeared normal upon inspection and palpation. The postoperative course was uneventful. At operation of patient no. 2 an encapsulated, partly calcified, tumor with a diameter of 20 mm was found in the ventro-caudal portion of the pancreatic head. The tumor was removed. The tail of the pancreas was palpated normal contradictory to the angiographical findings. No abnormality was found in other abdominal organs (including the liver and lymphnodes). The postoperative course was uneventful.

After operation both patients gained weight, showed normal glucose tolerance tests and their skinrash disappeared. One year after the operation both patients are without any signs of residual or recurrence.

Tissue glucagon: The glucagon concentrations in the two tumors were 82 microgram per g wet weight of tumor (patient no. 1) and 10 microgram per g wet weight of tumor (patient no. 2). Non-afflicted pancreatic tissue from patient no. 1 contained 1.2 microgram per g wet weight. The specimen from patient no. 2 consisted largely of calcified tissue, which explains the relatively low value.

Immuncytochemistry: The tumor of patient no. 1 contained numerous cells that displayed intense reactivity toward the glucagon antiserum (fig. 5). No gastrin, HPP or VIP immunoreactive cells were found. In a localized portion of the tumor numerous insulin-immunoreactive cells were present. Some, but not all of the insulin cells also contained glucagon. This unusual feature will be dealt with in a later report. From patient no. 1, pancreatic tissue outside the tumor was also available. Interestingly, glucagon cells were very few and showed poor immunoreactivity. Many islets actually lacked glucagon cells. Otherwise, the islets appeared normal with respect to insulin and HPP cells. The tumor of patient no. 2 was very sclerotic containing strands of compressed endocrine-like cells (fig. 6). In some areas necrosis was evident. A few, strongly immunoreactive glucagon cells could be found in the tumor which also contained a few VIP-immunoreactive cells. No gastrin, HPP or insulin immunoreactive cells could be demonstrated.

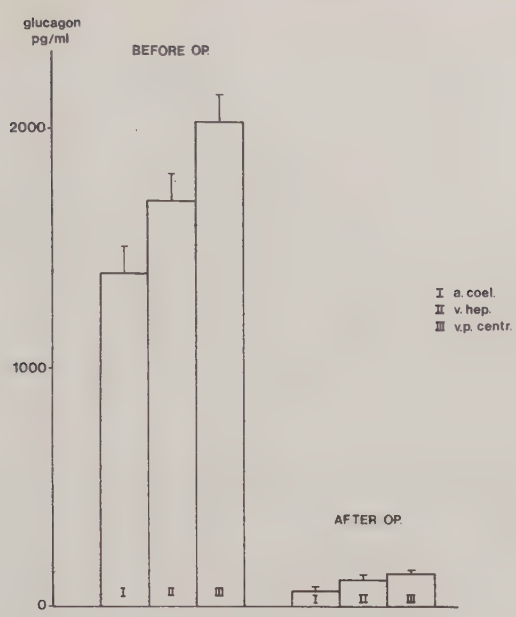

Fig. 2 a. Glucagonconcentration (pg/ml) in patient no. 1 before and after the operation. I = coeliac artery, II = main hepatic vein, III = main portal trunc.

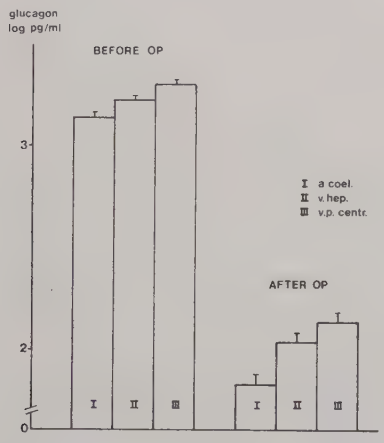

Fig. 2 b. Log. glucagonconcentrations from fig. 2 a in patient no. 1 before and after the operation. I = coeliac artery, II = main hepatic vein, III = main portal trunc.

		Glucagon pg/ml	/	Insulin µU/ml
1.	v. lienalis 1.	2900	/	6
2.	v. lienalis 2.	1880	/	4
3.	v. lienalis 3.	1640	/	4
4.	v. porta	1930	/	4
5.	v. aspd	1420	/	7
6.	g. c. t.	1250	/	3
7.	v. mes. sup	1320	/	3
8.	v. pspd	1500	/	34
9.	v. pancreat. dist. 9	24000	/	5
10.	v. lienalis hilus	2160	/	2
11.	v. lienalis dist.	1580	/	3
12.	v. lienalis dist. 12	1540	/	1
13.	v. lienalis dist. 13	2210	/	6
14.	v. porta centr.	2400	/	3

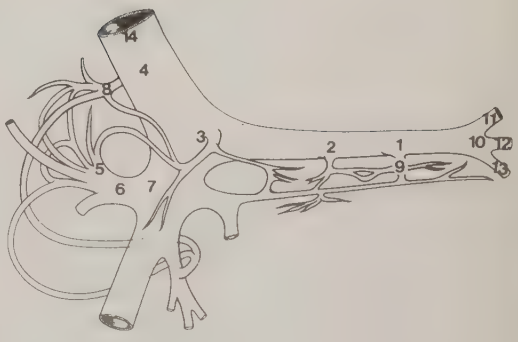

Fig. 3 a. Preoperative glucagon and insulin concentrations in different portal branches and pancreatic veins in patient no. 1.

		Glucagon pg/ml	/	Insulin µU/ml
1.	v. pancr.	350	/	100
2.	v. port. confl.	104	/	20
3.	v. lienal. dist.	470	/	80
4.	v. mes. sup.	50	/	3
5.	v. mes. sup. (outside GCT)	72	/	4
6.	v. port. centr.	114	/	12
7.	v. port. centr.	86	/	10
8.	v. port. centr.	110	/	8
9.	v. PSPD	156	/	26
10.	v. lienal. dist.	570	/	53
11.	v. port. centr.	160	/	8
12.	v. port. centr.	156	/	9
13.	v. port. centr.	174	/	7

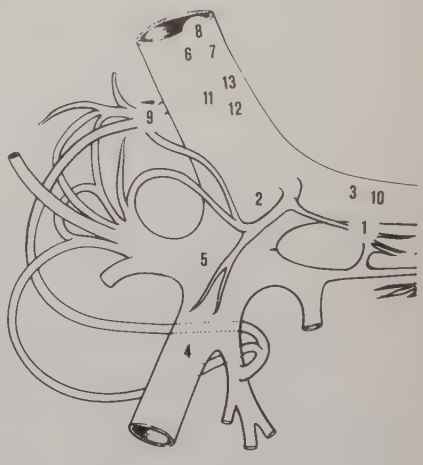

Fig. 3 b. Postoperative glucagon and insulin concentrations in different portal branches and pancreatic veins in patient no. 1.

		Glucagon pg/ml	/	Insulin µU/ml
1.	v. lienal.	= 950	/	150
2.	v. pancreatis (corpus)	= 1 200	/	300
3.	v. pancreatis (corpus)	= 680	/	330
4.	v. lienal. prox.	= 550	/	200
5.	v. lienal. prox.	= 680	/	65
6.	v. port. - mes. sup. - lienal. confluens	= 1 220	/	41
7.	v. mes. sup. (below GCT)	= 600	/	5
8.	v. PSPD	= 3 850	/	29
9.	v. PSPD	= 5 750	/	60
10.	v. lienal. dist. caudal	= 1 300	/	2
11.	v. lienal. dist.	= 1 050	/	21
12.	v. pancreat.	= 620	/	45
13.	v. pancreat.	= 1 150	/	53
14.	v. pancreat.	= 1 480	/	39
15.	v. mes. inf.	= 1 490	/	3
16.	v. ASPD	= 7 700	/	60
17.	v. ASPD	= 10 000	/	69
18.	v. GCT	= 800	/	7
19.	v. pancreat. (cauda)	= 850	/	41
20.	v. port. centr.	= 1 600	/	22
21.	v. port. centr.	= 1 750	/	25
22.	v. port. centr.	= 1 600	/	28

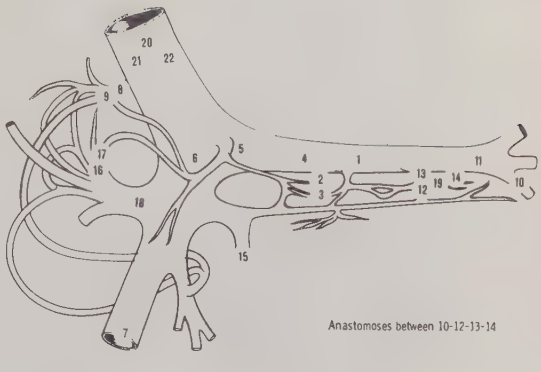

Fig. 4 a. Preoperative glucagon and insulin concentrations in different portal branches and pancreatic veins in patient no. 2.

		Glucagon pg/ml	/	Insulin µU/ml
1.	v. porta centr.	= 450	/	6
2.	v. gastro-epiploica sin.	= 330	/	5
3.	v. lienal. dist.	= missed	/	missed
4.	v. mes. inf.	= 350	/	5
5.	v. PSPD	= 116	/	missed
6.	v. PSPD	= 150	/	10
7	v. PSPD	= 150	/	11
8.	v. colica dx. (with anastomosis to GCT)	= 160	/	6
9.	v. mes. sup.	= 142	/	missed
10.	v. mes. sup. dist.	= 120	/	5
11.	v. pancreat. (cauda)	= 1 380	/	190
12.	v. lienal.	= 170	/	5
13.	v. lienal.	= 170	/	4
14.	v. lienal.	= 182	/	11
15.	v. lienal.	= 168	/	missed
16.	v. porta (outside v. PSPD)	= 176	/	12
17.	v. porta (prox. v. PSPD)	= 200	/	missed
18.	v. mes. sup. dist.	= 168	/	5

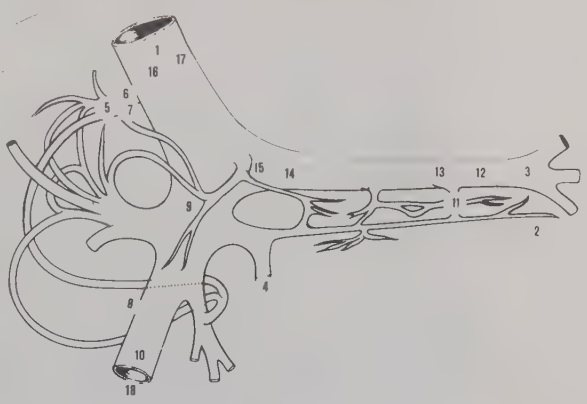

Fig. 4 b. Postoperative glucagon and insulin concentrations in different portal branches and pancreatic veins in patient no. 2.

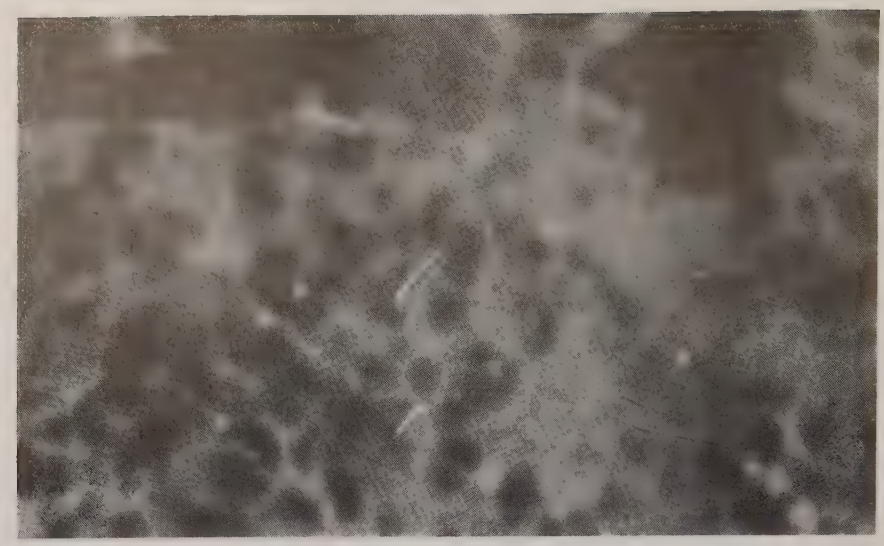

Fig. 5. Pancreatic tumor, patient no. 1. Glucagon immunofluorescence. The tumor forms ribbons and strands consisting almost entirely of strongly immunoreactive glucagon cells. x 490.

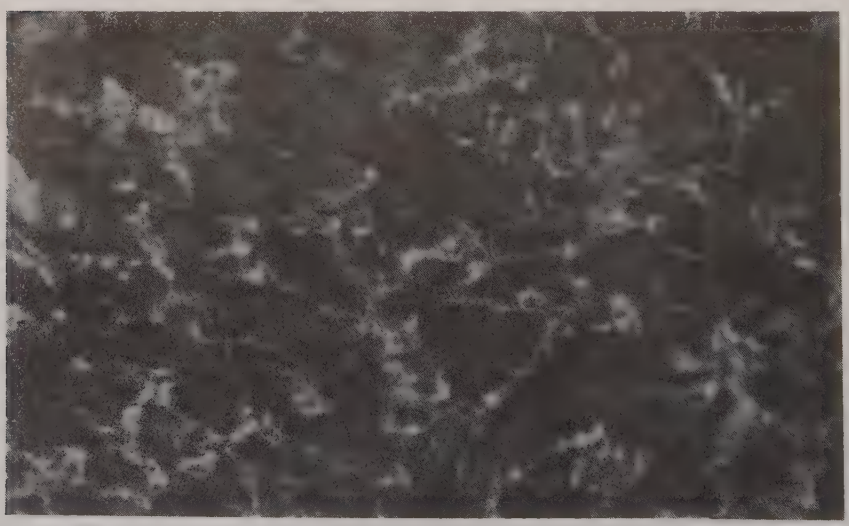

Fig. 6. Pancreatic tumor, patient no. 2. Glucagon immunofluorescence. In this very sclerotic tumor the glucagon cells are found compressed in between large masses of connective tissue. x 160.

Discussion

The role and potential use of arteriography in the diagnoses of insulinomas has recently been discussed (22). The aim of arteriography is to provide the surgeon with a preoperative localization. Small tumors are not found with this method and because of their small size are also liable to pass undetected at operation. Thus extensive damage to the pancreas may be caused during the search for the tumor and sometimes, when the tumor cannot be found, a blind pancreatic resection is performed (17, 22). Thus, there is a need for more accurate methods to localize endocrine pancreatic tumors. Vein catheterization with blood sampling for hormone assay has successfully been used for localization of phaeocromocytomas (2, 5) and parathyroid adenomas (20, 25). We have previously reported on the use of aorta, cava and pancreatic vein catheterization for preoperative localization of insulinomas (8, 9), islet cell hyperplasia (9), glucagonomas (7,9) and gastrinomas (9). With vena cava, abdominal aorta and portal catheterizations we demonstrated in these two patients that the tumors secreted glucagon to the portal venous system. The tumors were shown to drain through local pancreatic veins, thus excluding extrapancreatic tumors. We were also able to show which portion of the pancreas the tumors were localized to. This localization could be verified by palpation and visual inspection at operation. In patient no. 2 the glucagon levels in v. ASPD and v. PSPD were pathologically raised suggestive of two tumors. Pancreatic phlebography showed anastomosis between v. ASPD and v. PSPD (fig. 1 b, c). As the highest values were found in the v. ASPD the tumor was expected to drain through the v. ASPD but with anastomotic drainage through the v. PSPD. During operation a tumor was detected in the pancreatic portion mainly drained through the v. ASPD whereas none was found in the area mainly drained by the v. PSPD. The results of postoperative catheterization indicated radicality (fig. 4 b). In patient no. 1 preoperative portal phlebography showed suspected metastases in the right liverlobe. Neither arterio-venous hormone difference (glucagon and insulin) nor palpation or inspection during operation could localize any liver metastases wherefore false positive portographic findings were suspected. Mixed pancreatic endocrine tumors, as in patient no. 1, can cause deposits of only one of the endocrine cells (11). Normal glucagon concentrations across the liver could be present even with liver metastases if due to insulin secreting tumors.

Hyperglucagonemia is known to cause hyperglycemia. The high insulin concentration in the veins draining the pancreatic body of patient no. 2 (fig. 4 a) might be a compensatory hypersecretion in order to adjust the blood glucose level to normal values.

During the preoperative investigation of patient no. 2 a slow insulin decrease was seen in peripheral blood from 10 to 5 microunits per ml. P-glucose increased in coeliac artery samples from 6.4 mmol/l to 8.0 mmol/l. It seems likely that this pattern of response could be caused by an increased activity in the sympatho-adrenal system.

Postoperative catheterization assessed the radicality of operation in both patients.

Knowledge of the glucagon syndrome and availability of assays for glucagon will no doubt facilitate early recognition of glucagonomas. Raised glucagon concentrations in peripheral blood may be caused by tumors too small to be localized by arteriography and/or palpation peroperatively. The catheterization technique has proven itself able to localize hyperplasias and small non-palpable endocrine pancreatic tumors (9).

Conclusions

In the present two cases glucagon-secreting tumors were localized preoperatively by selective pancreatic vein catheterization and hormone assay. The high arteriovenous glucagon differences were caused by tumor tissue containing numerous glucagon cells and high tissue glucagon concentrations. After tumor removal the glucagonoma syndrome disappeared in both patients. Postoperative investigation assessed the success of operation. No complications were seen. The technique described seems very accurate and is proposed for use pre- and postoperatively in all patient with clinical and laboratory findings suggestive of endocrine pancreatic tumors.

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